TUMOR IMPRINTS AS A SOURCE OF NUCLEAR SUBSTRATE FOR THE DETECTION OF ANTINUCLEAR FACTORS*

THOMAS K. BURNHAM, M.B., B.S. (LONDON),† GERALD FINE, M.D.‡ AND THOMAS R. NEBLETT, PH.D.‡

To elucidate further the immunologic significance of our recent report of gamma globulin localization in skin lesions of lupus erythematosus and various dermatoses (1), we studied the sera of patients with positive lupus erythematosus cell tests, scleroderma and various dermatoses for antibodies to skin components by the indirect fluorescent technic.

Several scleroderma sera produced small bright yellow-green fluorescent speckles which appeared to be in epidermal cell nuclei (Fig. 1). To determine whether this could represent the "speckled" nuclear fluorescence described by Beck (2), we treated various tissues with these sera. We confirmed that this was indeed the previously described "speckled" nuclear fluorescence and found that tumor imprints were far superior to preparations using normal cells as nuclear substrate for the detection of antinuclear factors.

MATERIALS AND METHODS

Clinical Material

Sera of normal individuals and of patients with scleroderma, positive lupus erythematosus cell tests and various dermatoses were investigated.

Sections were cut in a cryostat at −15° C. six microns thick from quick-frozen surgically removed tumors, liver, kidney, spleen and involved, uninvolved and normal skin. Touch imprints were made from 13 different tumors (see Table I) and from normal tissues which included liver, spleen, thymus, lymph node and testis. The imprints were air-dried and stored at minus 15° C. Autopsy imprints were also studied. Bone marrow and buffy coat peripheral blood smears were treated similarly.

Staining and Examination

Specimens were incubated 30 minutes with serum on a slowly rotating shaker at 37° C., washed with saline and water, dried, and then covered for 30 minutes with commercially available fluorescein conjugated goat antihuman gamma globulin.§ This had been diluted 1:5 with buffered saline, pH 7.3, and extracted once with ethyl acetate. Rhodamine labelled bovine serum albumin, 0.05 cc in 2 cc of the diluted conjugate, was used as a counterstain. The stained slides were examined with a Leitz Ortholux microscope using an Osram L2 lamp, a BG 12 excitation filter and a pale yellow barrier filter (Leitz). Known negative and positive control sera were used with every procedure.

RESULTS

Specific nuclear fluorescence was bright yellow-green while cytoplasm stained orange with the rhodamine.

TABLE I

Nuclear speckles and tumor imprints

Tumors	Size of Speckles
Seminoma	+++
Leiomyosarcoma	+++
Choriocarcinoma	+++
Rhabdomyosarcoma	++++
Carcinoma of breast (2 different tumors)	+++
Papilloma of choroid plexus	+
Oligodendroglioma	++++
Carcinoma of lung	+++
Osteogenic sarcoma	+++
Melanoma	++
Hodgkins disease (2 different tumors (1) and (2))	(1) + (2) +++

\+ = Size of speckles in lymphocytes and other normal tissues.

++++ = About 4 × size of speckles in lymphocytes and other normal tissues.

Skin

Several scleroderma sera showed predominantly nuclear, bright yellow-green fluorescent speckles in the cells of the epidermis and hair follicles of involved and normal skin (Fig. 1). Cytoplasmic location of certain speckles was thought to be caused by nuclear material displaced when the sections were cut. Nucleolar fluorescence of epidermal cells of involved and normal skin was seen with one scleroderma serum.

Several of the L. E. cell positive sera produced homogeneous nuclear fluorescence in normal epidermis and hair follicles.

Other Tissues

Speckled nuclear fluorescence was seen in all the specimens (Figs. 2, 3, 4). The nuclear speckles

* From the Department of Dermatology† and the Department of Clinical Laboratories,‡ Henry Ford Hospital, Detroit, Michigan.

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§ May be purchased from Baltimore Biological Laboratories.

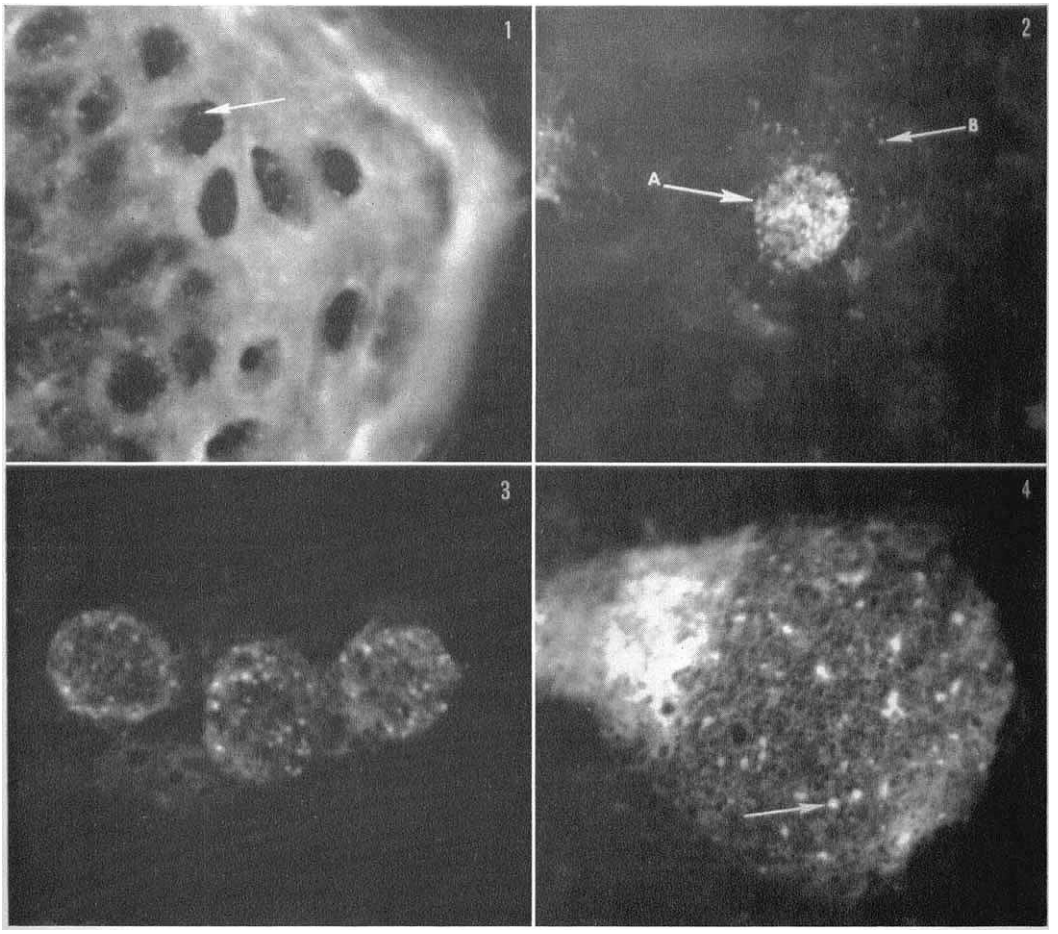

FIG. 1. Normal skin incubated with scleroderma serum. Arrow shows one of the nuclear speckles.
FIG. 2. Buffy coat peripheral blood smear incubated with scleroderma serum. A. nonspecific fluorescent cytoplasmic granules of polymorphonuclear leukocyte, also present when normal serum was used. B. small faint nuclear speckles.
FIG. 3. Leiomyosarcoma imprint incubated with scleroderma serum. Many bright large nuclear speckles are seen. Compare with the small faint speckles in Figure 2.
FIG. 4. Rhabdomyosarcoma imprint incubated with scleroderma serum. Arrow shows one of the bright large nuclear speckles. Compare with the small faint speckles in Fig. 2.
All photos were taken using 95 × Fluorite objective 10 × ocular, Leica MI body.

were brighter, more numerous and usually larger in the tumor imprints than in the other specimens (see Table I). Certain cells in the bone marrow smears, lymph node imprints and spleen sections also had larger speckles.

The tumor sections, although showing the same type of speckles, were not as good as the imprints due to nonspecific stromal fluorescence and poor cell delineation.

Nucleolar fluorescence as described by Beck (3, 4) was also much more conspicuous in the tumor imprints, with again brighter, larger and more numerous nucleoli.

Homogeneous and membranous nuclear fluorescence (Beck (4) were demonstrated much better in tumor, spleen and lymph node imprints than in blood smears or tissue sections due to the greater number, delineation and size (in tumor imprints only) of the nuclei.

Controls

No specific yellow-green nuclear fluorescence was seen in the control sera stained specimens.

EXPERIMENTAL VARIABLES

Tumor imprints were stored at −15° C. for up to 4½ months without loss of nuclear fluorescence potential. A group of imprints stored at room

temperature for five months failed to demonstrate nuclear fluorescence potential.

No loss of nuclear fluorescence potential occurred when tumors were stored for up to 96 hours at 4–7° C. prior to being imprinted.

Autopsy material imprints also demonstrated good nuclear fluorescence.

DISCUSSION

Tumor nuclei apparently contain more "speckled" and "nucleolar" antigens than normal nuclei, and therefore demonstrate these types of fluorescence more conspicuously. Homogeneous and membranous nuclear fluorescence are also seen much more readily in tumor and some tissue imprints than in blood smears or tissue sections due to the greater number, delineation and size of the nuclei. Imprints lack the nonspecific stromal fluorescence of sections and consequently have superior nuclear definition. Many slides can be prepared rapidly and stored for at least 4½ months. Tumor imprints are thus an ideal nuclear substrate for the detection of antinuclear factors.

SUMMARY

Sera of patients with positive lupus erythematosus cell tests, scleroderma and various dermatoses were investigated for antibodies to skin components and antinuclear factors by the indirect fluorescent antibody technic.

Speckled nuclear fluorescence in the epidermis was seen with several of the scleroderma sera. Epidermal nucleolar fluorescence occurred with one scleroderma serum, while homogeneous epidermal nuclear fluorescence was seen with several of the lupus erythematosus cell positive sera.

Tumor imprints were far superior for the detection of antinuclear factors to the conventional blood smears and tissue sections. Nuclear fluorescence was much more conspicuous due to the greater number, size and delineation of nuclei and to the greater quantity of some of the nuclear antigens. We therefore suggest the routine use of tumor imprints as a rapid and practical screening test for antinuclear factors.

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